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(54) Title: VACCINE FOR THE PROTECTION OF A VERTEBRATE ANIMAL AGAINST FUNGAL SKIN INFECTION

(57) Abstract: A vaccine for the protection against fungal skin infections, such as dermatophytic infections in animals, including ringworm. The vaccine comprises an immunostimulating material of a fungal species that is non-pathogenic to the animal and an immunologically amount of a fungal material that, in combination with the immunostimulating fungal material, confers immunity to fungal skin infections. The animals that can be protected include fur bearing animals including mink, foxes, chinchillas, minks, rabbits, martens, guinea pigs and racoon dogs and ruminants, swine and horses. The vaccine may contain material of *Trichophyton* species, *Microsporum* species, *Keratinomyces* species and *Epidermophyton* species.

VACCINE FOR THE PROTECTION OF A VERTEBRATE ANIMAL AGAINST FUNGAL SKIN INFECTION

FIELD OF THE INVENTION

The present invention relates in its broadest aspect to the field of protecting animals against infectious diseases and in particular there is provided vaccines comprising as an immunostimulating material, live material of a fungal species. More specifically, the invention provides vaccines or vaccine kits that confers protection against fungal skin infections in vertebrate animals. The vaccines according to the invention are especially useful for the prophylaxis and/or treatment of dermatophytosis (ringworm).

BACKGROUND OF THE INVENTION AND PRIOR ART

Fungal skin infection or skin mycosis (dermatomycosis) is an infection of keratinised tissue such as hair, nails and the stratum corneum of the skin of animals and humans. The most well known types of fungal skin infection are dermatophytoses, or ringworm, which is caused by a group of keratinophilic, parasitic fungi generally known as "dermatophytes" or "ringworm fungi". These belong to the genera *Trichophyton*, *Microsporum*, *Epidermatophyton* and *Keratinomyces*. An important characteristic of the dermatophytes is their restriction to dead keratinised tissues, and thus, in contrast to some other fungi, they are not able to cause systemic infection. There are also other fungi, such as *Malassezia*, *Phaeoannellomyces* and *Candida* species, that can cause fungal skin infection.

Although the dermatophytic fungi are not generally lethal, dermatophytic infection in the skin of humans and animals is often associated with stuntedness and general discomfort. In addition, secondary bacterial infections are often observed in the infected areas.

The clinical manifestation of dermatophytosis in for example fur bearing animals is, at an early clinical stage, that the guard hair is sticking out slightly in contrast to the undercoat. This is followed by hair loss, which gradually spreads outwards, and thickening of the skin and the build-up of a substantial crust. Histological examination has shown that the infection only affects the superficial layers of the skin, i.e. epidermis and dermis. The clinical

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changes of the skin, i.e. encrustation, clear from the centre and outwards, often leaving a lasting skin damage in the form of scar tissue and collagen deposits.

Thus, it is often seen that e.g. hides from cattle which have been infected with ringworm are scarred. Such hides will be worth less than undamaged hides, as the ringworm damage is readily seen on the leather after tanning.

In addition to the damage of hides, the incidence of dermatophytic infections in animals can cause serious consequences for animal owners. Thus, restrictions can be imposed on sale of animals infected with dermatophytes and exclusion from competitive events, such as e.g. horse races. Infected cats, dogs and livestock are often banned from show arenas, and national authorities may refuse export and import of such animals.

Another problem is that ringworm can be transmitted to humans, particularly ringworm in cattle, cats and dogs caused by e.g. *M. canis* and other dermatophytes, i.e. the disease has a zoonotic character. The dermatophytes are transmitted by the movement of infectious hairs or scales directly or indirectly from animals to humans, and hence causing a serious health risk for humans as the infection can necessitate long-term medical treatment and have social implications. Secondary infections can result in permanent scarring and other implications.

Presently, treatment of dermatophytosis in animals and humans includes topical therapy by antimycotics such as iodophors, various imidazole derivatives and griseofulvin. However, the development of lasting immunity, due to the initial infection, after the use of such external antimycotics has not been documented and thus medicament treatment of dermatophytosis cannot prevent reinfection. In addition, these chemical substances can have considerable side effects such as allergic reactions. It is therefore desirable to provide a vaccine against ringworm to prevent infection.

Immunotherapy by therapeutic use of vaccines against ringworm has been described. Mainly the use of inactivated strains of different dermatophytes has been disclosed. Thus, as an example, EP 0 393 371 discloses inactivated ringworm vaccines comprising dermatophytes of the genera *Trichophyton*, *Epidermophyton*, *Keratinomyces* and *Microsporum*, and US 5277904 discloses a dermatophyte vaccine comprising a suspension of killed der-

matophytes of the genera *Trichophyton* and *Microsporum* combined with an adjuvant compound.

The use of live, non-attenuated vaccines for the protection against dermatophytosis has been disclosed. Thus, US 4.368.191 describes a vaccine comprising live microconidia from a *Trichophyton mentagrophytes* strain for the prophylaxis and treatment of dermatophytosis caused by the dermatophyte *T. mentagrophytes*. RU 2084241 describes a live vaccine against Microsporum infection, which comprises microconidia of a *Microsporum canis* strain with low virulence. RU 2097065 discloses a live combination vaccine against ringworm caused by *Trichophyton sp* and *Microsporum sp*, comprising live microconidia from *Microsporum canis* and *Trichophyton equinum*, and RU 2084240 discloses a live vaccine against dermatophytosis comprising live microconidia from strains of *Microsporum canis* and *Trichophyton mentagrophytes*.

Inactivated vaccines are not always effective against dermatophytosis. In general inactivated vaccines often do not give efficient immunity, which is believed to be due to failure of such vaccines to elicit a sufficient cell-mediated immune response, e.g. the recruitment of helper T-cells. Inactivated vaccines tend to only mediate an antibody response, i.e. a humoral immunological response, which often is not sufficient to successfully protect against fungal infections.

In an experimental challenge trial (Bratberg et al, 1999, Annual members meeting AAVD & ACVD, 15. Proceedings 1999 Maui, Hawaii, Dermatology Investigative Studies (Abstracts) page 113-114), the efficacy of two commercial bovine ringworm vaccines was compared. One of them was an inactivated vaccine comprising three fungal species (*T. verrucosum*, *T. mentagrophytes* and *T. sarkisovii*) and the other was a live vaccine containing attenuated *T. verrucosum*. The conclusion from this experiment was that calves vaccinated with the inactivated vaccine developed severe ringworm lesions, whereas non of the calves vaccinated with the live vaccine developed ringworm signs.

Vaccines comprising live dermatophytes are well known for their ability to elicit both cell-mediated immune reactions and humoral responses. However, the preparation and handling of live vaccines can involve some difficulties and only strains which are safe and non-virulent, i.e. harmless and not pathogenic to the vaccinated animals or to humans, can be used. In addition to being of low virulence, live vaccines have to be immunogenic and to

be genetically stable. Moreover, known live vaccines are mostly limited to the prevention of infection caused by the homologous fungal species on which the vaccine is based, as cross-immunity to or resistance against infection by heterologous pathogenic fungal species or genera is rarely seen. These circumstances make it difficult to develop broad spectrum live vaccines which meet the above general requirements of being effective and safe for handlers and animals in contact with the vaccine.

However, it has now been found that a vaccine comprising, as an immunostimulating material, material of a fungal species that is non-virulent or non-pathogenic to the animal to be protected, in combination with antigenic material of a fungal species, is capable of conferring, in combination with the immunostimulating fungal material, immunity against fungal skin infections. Thus, the vaccines according to the present invention are especially useful for the prophylaxis and/or treatment of fungal skin infections, including dermatophytosis.

The present invention rests on the discovery that fungal material can be used in a vaccine as a very efficient immunostimulating agent which enhances the immunogenic response to the antigens towards which an immune response is required. It is contemplated that the effect of the immunostimulating fungal material is due to a general enhancement of the immune response in the animal and/or a better and more effective presentation of the antigens in the animal which is vaccinated. The vaccine according to the invention thus gives an improved protection against fungal skin infections as compared to similar vaccines without such a immunostimulating fungal material. It is also contemplated that this immunostimulating effect of fungal materials is a general effect which can be applied in general for formulating vaccine compositions having an enhanced efficacy as compared to vaccines not comprising the immunostimulating fungal material.

SUMMARY OF THE INVENTION

Accordingly, the present invention relates in a first aspect to a vaccine or vaccine kit for the protection of a vertebrate animal against fungal skin infection, said vaccine or kit comprising (i) an effective amount of an immunostimulating material of at least one first fungal species that is non-pathogenic to the vertebrate animal and (ii) an immunologically effective amount of material of at least one second fungal species that, in combination with

said material of a first fungal species, is capable of conferring immune protection in the animal against the fungal skin infection.

In other aspects there is provided a fungal strain which is selected from the group consisting of *Trichophyton equinum* strain DSM No. 13018, *Microsporum canis* DSM No. 13016, and *Microsporum canis* DSM No. 13017.

In a further aspect, there is provided a method of manufacturing a vaccine or kit according to the invention, the method comprising selecting an immunostimulating material of at least one first fungal species that is not pathogenic to the vertebrate animal, selecting at least one second fungal species which, in combination with said at least one first fungal species, is capable of conferring immune protection in said animal against said fungal skin infection, and combining said at least one first fungal species and said at least one second fungal species to obtain the vaccine or the kit.

In a still further aspect there is provided a method of preventing or curing a fungal skin infection in a vertebrate animal, the method comprising administering to the animal an effective dosage regimen of the vaccine or kit according to the invention.

In another aspect there is provided a method of producing an inactivated material of a fungal species that is capable of conferring immune protection in a vertebrate animal against a fungal skin infection, the method comprising a step of subjecting live material of said fungal species to a heat treatment at a temperature and for a period of time that is sufficient to inactivate the fungal material substantially without destroying its antigenic properties.

In a still further aspect the invention relates to a vaccine or vaccine kit for the protection of a vertebrate animal against an infectious disease, said vaccine or kit comprising (i) an effective amount of an immunostimulating material of at least one fungal species that is non-pathogenic to the vertebrate animal and (ii) an immunologically effective amount of an antigenic material of at least one species of a pathogenic organism that, in combination with said material of the at least one fungal species, is capable of conferring immune protection in said animal against said infectious disease.

DETAILED DISCLOSURE OF THE INVENTION

It is one primary objective of the invention to provide a vaccine or a vaccine kit as defined above for the protection of a vertebrate animal against fungal skin infection.

As used herein, the term "kit" refers to a presentation form comprising at least two separate components which in combination provides a vaccine composition that is ready for use. Thus, a kit according to the invention may comprise two or more separate fungal material preparations and, if required, one or more units of diluents, suspension media or other vehicles for any of the fungal components of the ready-to-use vaccine.

In the present context, the term "fungal skin infection" refers to any infection of the epidermis including its adnexal structures and the dermis, with any fungal species. Thus, such fungal skin infections include infections of the stratum corneum i.e. the dead keratinised tissue layer of the epidermis, subcutaneous infections, infections of hair and nails, and deep skin infections. Thus, as used herein, the term "fungal skin infection" includes conditions referred to as ringworm, skin mycosis, dermatophytosis, dermatomycosis, trichophytosis and microsporia. Also conditions such as Onychomycosis, Piedra, Pityriasis versicolor, Tinea barbae, Tinea capitis, Tinea corporis, Tinea cruris, Tinea favosa, Tinea nigra, Tinea pedis and Tinea unguium are included in the term "fungal skin infection".

The vaccine or the kit according to the invention comprises as one component an immunostimulating material of at least one fungal species which is non-pathogenic to the vertebrate animal to be vaccinated. Such a fungal material is preferably fungal propagules, i.e. fungal material which is viable and able to proliferate. In a presently preferred embodiment of the present invention, microconidia are applied, however it is contemplated that also macroconidia and other types of spores and hyphae can be used. In accordance with the invention, the fungal material has to be safe to use in a vaccine, and hence not pathogenic to the vertebrate animal. Furthermore, the immunostimulating fungal material should not pose any health risk to human beings or animals, and it should be genetically stable in order to avoid later development of virulent fungal strains. Whereas it is presently preferred that the immunostimulating material is a live or viable material, it is contemplated that at least certain fungal materials will have an immunostimulating effect in a non-viable state or inactivated state and accordingly, be useful in the present invention

The immunostimulating material of the present invention is a fungal material which is capable of stimulating a non-specific immune response to an infectious agent such as a skin fungus. Hence, there is generally no antigenic relationship between the immunostimulating material and the infectious agent. In the present context, fungal material which has this capability is denoted "immunostimulating material".

As mentioned above, when the immunostimulating fungal material according to the invention is used in a vaccine it has preferably a very effective immunostimulating effect and enhances the immunogenic response against the antigens towards which an immune response is required. It is contemplated that the effect of the immunostimulating fungal material is due to a general enhancement of the immune response in the animal and better and more efficient presentation of the antigens in the recipient of the vaccine.

It will be understood that any immunostimulating fungal material according to the invention can be used. However, such material is advantageously obtained from a skin fungus which is known to be non-pathogenic to the animal to be vaccinated. Thus, as an example, fungi of the species *Trichophyton equinum* and *Trichophyton gallinae* mainly causes skin infection in horses and poultry, respectively, and thus these species are good candidates for selecting an immunostimulating fungal material which is not pathogenic to other vertebrate animal species. Thus in one embodiment, the vaccine according to the invention comprises an immunostimulating fungal material which is derived from a *Trichophyton* species, including *Trichophyton equinum*. A specific example of such strain is *Trichophyton equinum* (DSM No. 13018).

In addition to the above immunostimulating material of a fungal species, the vaccine of the present invention comprises an immunologically effective amount of a fungal species which is capable of conferring an immune protection against a fungal skin infection in the animal to be vaccinated, i.e. an immunogenic fungal species.

In accordance with the invention an "immunologically effective amount of at least one second fungal species" is an amount of the immunogenic fungus which, when administered together with the above immunostimulating material of at least one first fungal species, will provide an immune protection against the skin fungus causing the fungal skin infection. The immunogenically active fungal species is preferably in a form that is selected from a viable, but attenuated fungal material and an inactivated fungal material. As used

herein, the term "attenuated" refers to a state of the fungus where it is still viable, but has a reduced or eliminated virulence or pathogenecity as compared to a non-attenuated parent strain.

In a presently preferred embodiment of the invention, the fungal material from the at least one second fungal species is inactivated by heat treatment, however it is also contemplated that other forms of inactivation can be applied such as the use of chemical substances such as e.g. formaldehyde.

The most common pathogenic fungal species associated with fungal skin infection of animals and humans are dermatophytes of the genera *Trichophyton*, *Microsporum*, *Keratinomyces* and *Epidermophyton* causing dermatophytosis or ringworm. Thus different species of dermatophytes may cause clinical infections in animals and humans such as *Trichophyton gypseum*, *Trichophyton mentagrophytes, Trichophyton verrucosum* and *Microsporum canis*.

Accordingly, the immunologically effective fungal material of the second fungal species of the present invention can advantageously be derived from a dermatophyte which is selected from a *Microsporum* species, a *Trichophyton* species, a *Keratinomyces* species and a *Epidermophyton* species. These dermatophytes includes species such as *Microsporum audouini*, *Microsporum canis*, *Microsporum distortum*, *Microsporum equinum*, *Microsporum gypseum*, *Microsporum nanum*, *Trichophyton concentricum*, *Trichophyton equinum*, *Trichophyton gallinae*, *Trichophyton gypseum*, *Trichophyton megnini*, *Trichophyton mentagrophytes*, *Trichophyton quinckeanum*, *Trichophyton rubrum*, *Trichophyton schoenleini*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Trichophyton verrucosum var. album*, *Trichophyton verrucosum var. discoides*, *Trichophyton verrucosum var. ochraceum*, *Trichophyton violaceum* and *Epidermophyton floccusum*. Specific examples of such strains are *Microsporum canis* R1/96-3088 (DSM No. 13016) and *Microsporum canis* R2/96-3288 (DSM No. 13017).

However, also other pathogenic skin infecting fungi such as yeast may be associated with fungal skin infection. Thus, the vaccine or kit of the present invention may in useful embodiments comprise a fungal species selected from a *Malassezia* species, a *Phaeoannellomyces* species, a *Candida* species, including *Candida albicans*, a *Scopulariopsis brevicaulis* species and an *Aspergillus* species.

In accordance with the invention, the vaccine can comprise an effective amount of material of two or more fungal strains which are capable of conferring immune protection in the animal against fungal skin infection. It will be appreciated that such two or more fungal strains may be of the same or different species. The immunogenic fungal material can suitably be in the form of fungal propagules including microconidia, macroconidia or other types of spores, and hyphae.

It is contemplated that the immunogenic fungal material needs not be in a whole cell or hyphal form. Thus, any fragment or part of the fungal material that contains an antigenic moiety or epitope, such as e.g. surface proteins, toxins or fragments from disintegrated fungus material, may be applied successfully in the vaccine or the vaccine kit. The antigenic moiety can include a single epitope or a plurality of epitopes from a fungal species as long as at least one epitope is included which will produce a sufficient immune response to confer resistance to the fungal skin infection upon the recipient of the vaccine. Such fungal material comprising antigenic moieties may be prepared by any available means including homogenisation of a fungal material or parts thereof, disintegration of fungal material e.g. by high pressure, fractionation of fungal preparations, production of fungal material comprising at least one epitope by recombinant DNA technology, isolation of fungal secretions and the culturing of fungal material from fungal skin infection lesions.

Vaccines according to the invention can in useful embodiments confer protection against fungal skin infections in mammals, such as fur bearing animals, including *Canidae* species, *Felidae* species, mink, chinchilla, rabbit, marten, guinea pig and racoon dog, and mammals such as ruminants, horse, swine and man.

The vaccines of the present invention can be administered in the absence of additional immunostimmulatory substances. Therefore, in one embodiment, the vaccines of the present invention do not comprise adjuvants or other immunomodulatory or immunostimulatory substances than the immunostimulating fungal material.

However, to further increase their immunogenic properties of the vaccines of the present invention, the vaccines comprise, in another embodiment, at least one further immunostimulating component, preferably an adjuvant. Examples of suitable adjuvants include oil-in-water emulsions, water-in-oil emulsions, aluminum compounds including aluminium hydroxide, synthetic polymers, liposomes and cytokines.

The vaccine according to the invention can be stabilised by any suitable processing technique that are known *per se* in the art, such as e.g. by lyophilisation. When applying lyophilisation it may be useful to further apply a cryoprotective compound. The vaccine may also be in a form wherein one or both of the immunostimulating material and the immunogenic fungal material is suspended in a liquid phase such as e.g. an aqueous medium including a buffer and/or a salt solution.

The immunostimulating fungal material in a vaccine that is in a liquid form is preferably in the form of conidia being present at a number that is in the range of about 10^2-10^{10} microconidia per ml such as the range of about 10^3-10^8, such as the range of 10^4-10^7, including the range of 10^5-10^6 microconidia per ml. In other useful embodiments of a liquid vaccine, the immunogenic fungal material is present as microconidia, e.g. at a number in the range of about 10^2-10^{10} microconidia per ml, including the range of about 10^3-10^8, such as the range of 10^4-10^7, including the range of 10^5-10^6 microconidia per ml.

As mentioned above, a further aspect of the present invention is to provide a method of manufacturing a vaccine or a kit for the protection of a vertebrate animal against a fungal skin infection as defined above

The selection of the immunostimulating fungal material is preferably carried out in accordance with the above mentioned safety and efficacy criteria. Thus, the material should be selected so as to have weak or low virulence, absence of infection upon contact with animals or humans and should not cause diseases when injected into animals or humans. Furthermore, the fungal material should be genetically stable and retain its immunostimulating properties, e.g. during passages on animals.

The selection of the immunogenic fungal species as used in the vaccine of the invention, can be carried out by standard techniques well known in the art. These techniques generally comprise identification of the causative agent for the fungal skin infection, isolation and cultivation of this agent, finding of a method for reducing or eliminating, if required, the pathogenic effects without bringing about a loss of immunogenecity, e.g. by attenuation or inactivation and purifying the immunogenic fungal species or its products. It will be appreciated that the immunogenic fungal species may be a species that is not pathogenic to the animal species to be vaccinated, but which confers immunity against a different, but

pathogenic fungal species in the animal to be vaccinated, i.e. a species causing cross-immunity.

In a presently preferred embodiment, the immunogenic fungal species is inactivated prior to being combined with the immunostimulating fungal material. As mentioned above such inactivation can be done by any standard technique well known in the art.

It is well known in the art that it may be difficult to inactivate fungal material which is to be applied in vaccines in a manner which do not destroy its antigenic properties. Thus, treatment with chemical compounds and the subjection of fungal material to high temperatures may e.g. destroy cell wall proteins and thereby result in a loss of its immunogenecity.

However, it has surprisingly been found that immunogenic fungal material for use in vaccines can be efficiently inactivated by subjecting it to a heat treatment. Especially it has been found that heat treatment at a temperature and for a period of time that inactivate the fungal material, can be performed substantially without destroying the antigenic properties of the material. Preferably, the fungal material is subjected to a controlled heat treatment at a temperature within the range of about 40-60°C, including the range of about 45-55°C, such as the range of about 48-52°C, and preferably for a period of time within the range of about 30-1440 min such as the range of about 35-720 min, including 40-300 min, such as about 45-120 min, including about 60 min.

In one embodiment, the vaccines of the present invention can be used for preventing or curing a fungal skin infection in a vertebrate animal, including animals as mentioned above. The method of curing and/or preventing a fungal skin infection comprises the administration of an effective dosage regimen of the vaccine or kit according to the invention. Thus, the vaccines of the present invention can be administered parenterally, including by intramuscular injection, intravenous injection, intracutaneous injection, subcutaneous injection and topically, but they can also be administered by the oral route, when associated with a pharmaceutically acceptable solid or liquid vehicle.

It is a further objective of the invention to provide a method of producing an inactivated material of a fungal species that is capable of conferring immune protection in a vertebrate animal against a fungal skin infection. The method comprises a step of subjecting a

live fungal material to a heat treatment at a temperature and for a period of time that is sufficient to inactivate the fungal material substantially without destroying its antigenic properties. The heat treatment can advantageously be performed as defined above. In a preferred embodiment the fungal material to be inactivated is selected from a *Microsporum* species, a *Trichophyton* species, a *Keratinomyces* species and an *Epidermophyton* species.

As mentioned above there is also provided a vaccine or vaccine kit for the protection of a vertebrate animal against an infectious disease, said vaccine or kit comprising an effective amount of an immunostimulating material of at least one fungal species as defined above, and an immunologically effective amount of an antigenic material of at least one species of a pathogenic organism that, in combination with said material of the at least one fungal species, is capable of conferring immune protection in the animal against an infectious disease. In a presently preferred embodiment of the invention the at least one fungal species is a *Trichophyton* species such as a *Trichophyton equinum* including the strain DSM No. 13018.

Thus, it will be appreciated, that the vaccine according to the invention, in addition to the immunostimulating material of a fungal species, can comprise antigenic material from a pathogenic organism in an amount and form which is effective to confer immunity against an infectious disease. Such antigenic material can advantageously be derived from any type of pathogenic organisms, including material of a bacterial, viral, fungal or protozoan species, such as viable whole cells, attenuated whole cells, inactivated whole cells and parts of any of said species comprising at least one epitope.

The invention will now be further illustrated in the following non-limiting examples and the drawings wherein Fig. 1. shows the clinical signs of ringworm infection in guinea pigs challenged with *Microsporum canis*.

EXAMPLE 1

Preparation of a vaccine against skin infection with *Microsporum canis*

A strain of *Trichophyton equinum* designated R5/96 *T. equinum* - 2251 (DSM No.13018) was grown on a solid medium (Wort agar) for 21 days at 26°C. The fungal biomass was subsequently harvested using a spatula and transferred to a homogeniser. 200 ml sterile

distilled water was added and the resulting suspension was homogenised 2 × 1 min at maximum speed, and the homogenate was transferred to a sterile bottle with screw cap. Strict aseptic conditions were maintained throughout all handling. A 1 ml sample was withdrawn from the bottle in order to determine the number of spores by direct enumeration using a microscope, and to determine the number of CFUs (colony forming units). The CFUs were subsequently determined to be 1.2×10^9 CFU/ml and the number of spores determined by direct microscopic enumeration was 2.6×10^9 spores/ml.

Trichophyton equinum strain R5/96 *T. equinum* - 2251 was deposited under the Budapest Treaty on 18 August 1999 with DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany) under the accession No. DSM 13018.

Two strains of *Microsporum canis*, designated R1/96 *M. canis* - 3088 and R2/96 *M. canis* - 3288 were grown separately on a solid medium (Wort agar) for 20 days at 26°C. The fungal biomass was harvested, homogenised and transferred to two sterile bottles with screw cap each containing 200 ml of sterile distilled water, using the same procedure as described above. The number of spores in the resulting homogenates was determined in a 1 ml sample withdrawn from each homogenate by direct enumeration. The R1/96 *M. canis* - 3088 homogenate and the R2/96 *M. canis* - 3288 homogenate contained 9×10^8 spores/ml and 1.04×10^9 spores/ml, respectively.

R1/96 *M. canis* - 3088 and R2/96 *M. canis* - 3288 were deposited under the Budapest Treaty on 18 August 1999 with DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany) under the accession Nos. DSM 13016 and DSM 13017, respectively.

The two *M. canis* strains were inactivated by incubating the homogenates at 50°C for 24 h. The bottles were shaken several times during the incubation. In order to determine that the strains were inactivated, 2×0.5 ml of sample were withdrawn from the bottles and inoculated onto petri dishes with Sabouraud agar. No growth was observed after 21 days of incubation at 26°C, and thus the strains were 100% inactivated.

The above homogenates were mixed in order to give a final concentration of 200×10^6 spores/ml of each strain in the vaccine concentrate. Thus, 72 ml of R1 homogenate, 62 ml

of R2 homogenate and 26 ml of R5 homogenate were mixed in a beaker giving a total of 160 ml. To this was added 160 ml of protective medium (4 wt% gelatine, 20 wt% saccharose, distilled water, pH adjusted with 4M NaOH to pH 7.0-7.4, autoclaved for 20 min at 121°C, pH 6.2 - 6.8 in final medium). Aliquots of 5 ml of the vaccine were dispensed into 50 ml vials and lyophilised overnight.

The lyophilised vaccine was subsequently rehydrated and tested in order to determine the purity of the vaccine. The vaccine dissolved within 30 seconds and only growth from *T. equinum* was detected when cultivated on Trichophyton agar. After rehydration of the vaccine in 50 ml solvent, the total number of spores and number of live spores (CFU) were determined to be 5.6×10^7/ml and 4.4×10^6/ml, respectively.

EXAMPLE 2

Vaccination of silver foxes

The vaccine prepared and rehydrated as described in Example 1 was tested against ringworm infection in silver foxes.

Six silver foxes (*Vulpes vulpes)* from the same litter were vaccinated at the age of one month, with a dosage of 0.5 ml of the vaccine and again two weeks after the first vaccination using the same amount.

Five weeks after the second vaccination, the foxes, including a control group of 4 non-vaccinated foxes, were challenged epicutaneously using a *Microsporum canis* (R14/96) culture, by rubbing 0.5 ml of the culture into each of two 5 × 5 cm hairless fields along the spine, using a gloved finger. The culture had a concentration of 10×10^6 microconidia/ml, as determined by microscopic enumeration.

The results of the above experiment are summarised in the below Table 1

TABLE 1

Silver fox	Days after challenge						
	3	7	10	14	17	21	28
Non-vaccinated control foxes							
No. 1	-	-	(+)	++	++/+++	++	-
No. 2	-	(+)	+	+++	+++	+++	-
No. 3	-	-	+	+++	+++	+++	-
No. 4	-	(+)	+	+++	+++	+++	-
Vaccinated foxes							
No. 5	-	(+)	(+)	-	-	-	-
No. 6	-	(+)	(+)	-	-	-	-
No. 7	-	(+)	(+)	-	-	-	-
No. 8	-	(+)	(+)	-	-	-	-
No. 9	-	(+)	(+)	-	-	-	-
No. 10	-	(+)	(+)	-	-	-	-

- = no visible symptoms
(+) = a few small crusts approximately 1 mm in diameter
+, ++, +++ = increasing degree of ringworm lesions. From mild single lesions or crusts with thickening of the skin, to severe confluent lesion covering most of the inoculated site.

As can be seen from the above table 1, the foxes immunised with the vaccine did not evolve any sign of ringworm lesion and only a few small crusts of about 1 mm in diameter were observed in the inoculated area. This was only observed for the first 10 days after inoculation. After 14 days the crusts peeled of and no signs of inflammation or allergic reactions were observed.

The non-vaccinated control foxes all developed strong inflammatory reactions of the infected skin after 14 days as shown by the formation of many thick and solid crusts in the infected areas. These crusts peeled of after 21-28 days.

Thus, it is clearly seen from the above, that the vaccinated foxes were immunised against ringworm lesions caused by the experimentally induced *Microsporum canis* infection.

EXAMPLE 3

Vaccination of blue foxes

The vaccine prepared and rehydrated as described in Example 1 was tested against ringworm infection in blue foxes.

The experiment was conducted as described in Example 2, using 6 blue foxes (*Alopex lagopus*) vaccinated twice using 0.5 ml of vaccine, with 13 days between the first and second vaccination.

Seven weeks after the second vaccination, the foxes, including a control group of 4 non-vaccinated foxes, were challenged epicutaneously using a *Microsporum canis* (R14/96) culture as described in Example 2 using per animal 0.5 ml culture having a concentration of approximately 10×10^6 microconidia/ml.

The results of the above experiment are summarised in the below Table 2

TABLE 2

Blue fox	Days after challenge							
	3	7	10	14	17	21	28	35
Control foxes								
No. 16	-	(+)/+	(+)/+	+	+/++	++	(+)	-
No. 17	-	(+)/+	(+)/+	++	++	+++	+	-
No. 18	-	(+)	(+)	++	+++	+++	++	-
No. 19	-	-	(+)	(+)	+	+++	-	-
Vaccinated foxes								
No. 11	-	(+)	(+)/+	(+)	-	-	-	-
No. 12	-	-	(+)	-	-	-	-	-
No. 13	-	(+)	(+)	(+)/+	-	-	-	-
No. 14	(+)	(+)	(+)/+	(+)/+	+	(+)	-	-
No. 15	-	(+)	(+)	(+)	-	-	-	-
No. 20	-	-	(+)	-	-	-	-	-

- = no visible symptoms
(+) = a few small crusts approximately 1 mm in diameter
+, ++, +++ = increasing degree of ringworm lesions. From mild single lesions or crusts with thickening of the skin, to severe confluent lesion covering most of the inoculated site.

As can be seen from the above Table 2, the foxes which had received the vaccine did not develop ringworm lesions and only a few small crusts (less than 1 mm in diameter) were observed in the inoculated area. These small crusts were generally only observed for the first 14 days after inoculation. Except for fox No. 14, no clinical symptoms were observed in the infected areas 14-17 days after inoculation. Fox No. 14 had no clinical symptoms 28 days after inoculation.

In contrast hereto, the non-vaccinated control foxes developed strong inflammatory reactions 10-14 days after inoculation, and a heavy formation of thick and solid crusts was ob-

served in the infected areas of all the non-vaccinated foxes. After 35 days there were no signs of infection in the non-vaccinated foxes.

These results thus confirms the results presented in Example 2, as it is seen from the above, that the vaccinated foxes were immunised against ringworm lesions caused by experimentally induced *Microsporum canis* infection, whereas the non-vaccinated blue foxes all very heavily infected.

EXAMPLE 4

Vaccination of rabbits

The vaccine prepared and rehydrated as described in Example 1 was tested against ringworm infection in rabbits.

The experiment was conducted essentially as described in Example 2, using 5 rabbits vaccinated twice with a dosage 0.5 ml vaccine, with 14 days between the first and second vaccination.

The rabbits, including a control group of 3 non-vaccinated rabbits were experimentally challenged with *Microsporum canis* (R14/96) by rubbing 0.5 ml of culture liquid into a hairless field of the animals, using a gloved finger. The culture liquid had a concentration of approximately 10×10^6 *M. canis* microconidia/ml. The challenge was conducted approximately five weeks after the second vaccination.

The results of the above experiment are summarised in the below Table 3

TABLE 3

Rabbit	Days after challenge							
	5	7	11	14	18	21	28	35
Control rabbits								
No. 25	-	-	-	++	++	(+)	-	-
No. 26	-	(+)	+	++	++	+	-	-
No. 27	-	(+)	+	++	+	+	-	-
Vaccinated rabbits								
No. 20	-	-	-	-	-	-	-	-
No. 21	-	(+)	(+)	+/(+)	-	-	-	-
No. 22	-	-	-	-	-	-	-	-
No. 23	-	(+)	-	-	-	-	-	-
No. 24	-	(+)	-	-	-	-	-	-

- = no visible symptoms
(+) = a few small crusts approximately 1 mm in diameter
+, ++, +++ = increasing degree of ringworm lesions. From mild single lesions or crusts with thickening of the skin, to severe confluent lesion covering most of the inoculated site.

As can be seen from the above Table 3, non of the vaccinated rabbits did show any sign of ringworm infection. At the most there were a few small crusts 7-14 days after infection in the infected areas. However, these small crusts disappeared 18 days after infection, and no clinical symptoms were observed after this period of time.

It is also seen from Table 3 that the control group was heavily infected and showed strong signs of ringworm infection. Thus, 14 days after infection large and thick crusts were observed in the infected areas of all the non-vaccinated rabbits.

These results thus confirm the results presented in Example 2 and 3.

EXAMPLE 5

Vaccination of guinea pigs

The vaccine prepared as described in Example 1 was tested against ringworm infection in guinea pigs.

The lyophilised vaccine was rehydrated with two different volumes of sterile PBS to obtain to different concentrations of the vaccine. At the lowest concentration the total number of microconidia per dose was as estimated by microscopy 10×10^7. Each component consti-

tuted for one third of the total number (3.3×10^7). The number of live microconidia per dose was 10×10^6 (*T. equinum*). The highest concentration was 5 times the lowest concentration.

60 female guinea pigs (strain: HsdPoc:DH), 3 weeks old and a weight of 200-250 g were allocated into three groups, group 1, 2 and 3. Group 1 was injected twice with PBS (controls), group 2 was injected twice with the vaccine at the lowest concentration (normal dose) and group 3 was injected twice with the vaccine at the highest concentration (5 x normal dose). The two injections were given at 14 days interval.

Three weeks after the second injection, all guinea pigs were challenged epicutaneously using a *Microsporum canis* strain (K 1497/98) isolated from a cat with ringworm. The strain has a medium virulence for guinea pigs. Approximately 0.2 ml of a fungal suspension of the *M. canis*-strain containing approximately 0.8×10^6 macroconidia/ml was massaged on a shaved skin area of 5 x 3 cm with a sterile cotton swab. Three animals, one from each group, were stabled in each of 20 Macrolon cages (type IV).

The clinical symptoms were scored, using a numerical system ranging from 0 to 6, where: 0 is no symptoms; 1 is a few scales; 2 is reddening with swelling and modest scaling; 3 is reddening with swelling and a few small crusts; 4 is reddening with swelling and crusts; 5 is intensive production of crusts with wet inflammation; 6 is connected crusts with intensive inflammation of the skin under the crusts (reddening and swelling) and deep wet erosions.

In the healing phase of the disease, the symptoms were scored in the same way but in the opposite direction (from 5 to 0), where: 5 is beginning separation of crusts with wet inflammation; 4 is reddening with some crusts and scales; 3 is reddening with a few small crusts; 2 is reddening with scales and hairless skin or occurrence of new hair; 1 is a few scales; 0 is no symptoms.

The results of the above experiment are summarised in Table 4 and Figure 1.

TABLE 4

Days after challenge	Group					
	Controls		Normal dose		5 times normal dose	
	Score*	SD	Score	SD	Score	SD
3	0		0		0	
6	0		0		0.05	0.22
9	0.1	0.31	0		0.05	0.22
14	1.9	0.97	0.6	0.68	0.3	0.57
17	2.7	0.98	0.5	0.76	0.35	0.67
21	2.75	1.16	0.25	0.44	0.25	0.55
24	1.7	0.80	0.05	0.22	0.1	0.31
27	1.5	0.69	0.1	0.31	0.05	0.22
31	1.1	0.72	0.05	0.22	0	
34	0.5	0.89	0			
38	0.25	0.55				
41	0.1	0.31				

* Score: The clinical symptoms were scored, using a numerical system ranging from 0 to 6, where: 0 is no clinical symptoms of disease, and 1 to 6 is increasing degree of disease. See the text above for details.
SD = Standard deviation.

As can be seen from Table 4 and Figure 1, the average score in the control group is at a significant higher level than in both of the vaccinated groups in the period from day 15 to 31 after challenge. There was no significant difference between the two vaccinated groups. 21 out of 40 vaccinated guinea pigs did not show any clinical signs at all after challenge, and non of the remaining 19 animals had crusts (a score of 3 or more) at any time point during the observation period. 18 out of 20 control guinea pigs (90%) had crusts (a score of 3 or 4) at one or more time points.

No animals in the current study had scoring value of 5 and 6.

Thus, it is clearly seen from the above, that the vaccinated guinea pigs were immunised against ringworm caused by the experimentally induced *Microsporum canis* infection.

EXAMPLE 5

Production of a heat inactivated antigenic material of a fungal species

Fungal material from two strains of *Microsporum canis* (R1/96 *M. canis* - 3088 and R2/96 *M. canis* - 3288) was incubated at 50°C in order to investigate the time required to inacti-

vate the fungal material. At this temperature the inactivation is done substantially without destroying the antigenic properties of the fungal material. Fungal material from the two strains was incubated in flasks with screw cap and they were shaken at regular intervals. Samples were removed from the flasks at appropriate points in time and the number of CFUs was determined. As can be seen from the below Table 5, *M. canis* R2/96 was completely inactivated after only 3 hours at 50°C.

TABLE 5

Hours	0	1	2	3	4
CFU/ml	1×10^{8}	3.6×10^{4}	2×10^{2}	2×10^{0}	0

In a similar experiment *M. canis* R1/96 was inactivated completely after 1 h at 50°C, as no colony forming units were detected after this period of time.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 5, line 5.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

Deutsche Sammlung von Mikroorganismen und Zellkulturen, GmbH

Address of depositary institution *(including postal code and country)*

Mascheroder Weg 1b
D-38124 Braunschweig
Germany

Date of deposit	Accession Number
18 August 1999	DSM 13018

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

As regards the respective Patent Offices of the respective designated states, the applicants request that a sample of the deposited microorganisms only be made available to an expert nominated by the requester until the date on which the patent is granted or the date on which the application has been refused or withdrawn or is deemed to be withdrawn.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer: Yolaine Cussac	Authorized officer

INDICATIONS RELATING TO DEPOSITED MICROORGANISMS
(PCT Rule 12bis)

Additional sheet

In addition to the microorganism indicated on page 5 of the description, the following microorganisms have been deposited with

Deutsche Sammlung von Mikroorganismen und Zellkulturen. GmbH
Mascheroder Weg 1b
D-38124 Braunschweig
Germany

on the dates and under the accession numbers as stated below:

Accession number	Date of deposit	Description Page No.	Description Line No.
DSM 13016	18 August 1999	5	6
DSM 13017	18 August 1999	5	6

For all of the above-identified deposited microorganisms, the following additional indications apply:

As regards the respective Patent Offices of the respective designated states, the applicants request that a sample of the deposited microorganisms stated above only be made available to an expert nominated by the requester until the date on which the patent is granted or the date on which the application has been refused or withdrawn or is deemed to be withdrawn.

CLAIMS

1. A vaccine or vaccine kit for the protection of a vertebrate animal against fungal skin infection, said vaccine or kit comprising

(i) an effective amount of an immunostimulating material of at least one first fungal species that is non-pathogenic to the vertebrate animal and

(ii) an immunologically effective amount of material of at least one second fungal species that, in combination with said material of a first fungal species, is capable of conferring immune protection in said animal against said fungal skin infection.

2. A vaccine or kit according to claim 1 where the at least one first fungal species is a *Trichophyton* species.

3. A vaccine or kit according to claim 2 where the at least first fungal species is *Trichophyton equinum* including the strain DSM No. 13018.

4. A vaccine or kit according to claim 1 where the at least one second fungal species is in a form selected from the group consisting of viable, but attenuated fungal material and inactivated fungal material.

5. A vaccine or kit according to claim 4 where the at least one second fungal species is inactivated by heat treatment.

6. A vaccine or kit according to claim 1 where the at least one second fungal species is a dermatophyte.

7. A vaccine or kit according to claim 6 where the dermatophyte is selected from the group consisting of a *Microsporum* species, a *Trichophyton* species, a *Keratinomyces* species and a *Epidermophyton* species.

8. A vaccine or kit according to claim 7 where the dermatophyte is selected from the group consisting of *Microsporum audouini*, *Microsporum canis*, *Microsporum distortum*,

Microsporum equinum, *Microsporum gypseum*, *Microsporum nanum*, *Trichophyton concentricum*, *Trichophyton equinum*, *Trichophyton gallinae*, *Trichophyton gypseum*, *Trichophyton megnini*, *Trichophyton mentagrophytes*, *Trichophyton quinckeanum*, *Trichophyton rubrum*, *Trichophyton schoenleini*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Trichophyton verrucosum var. album*, *Trichophyton verrucosum var. discoides*, *Trichophyton verrucosum var. ochraceum*, *Trichophyton violaceum* and *Epidermophyton floccusum*.

9. A vaccine or kit according to claim 6 where the dermatophyte is selected from the group consisting of R1/96 *M. canis* - 3088 (DSM No. 13016) and R2/96 *M. canis* - 3288 (DSM No. 13017).

10. A vaccine or kit according to claim 1 where the at least one second fungal species is selected from the group consisting of a *Malassezia* species, a *Phaeoannellomyces* species, a *Candida* species, a *Scopulariopsis brevicaulis* and an *Aspergillus* species.

11. A vaccine or kit according to any of claims 1-10 comprising two or more second fungal species capable of conferring immune protection in said animal against fungal skin infection.

12. A vaccine or kit according to claim 1-11 where at least part of the at least one first fungal species is in the form of fungal propagules including microconidia and macroconidia.

13. A vaccine or kit according to claim 1 which confers protection against fungal skin infection in a mammal.

14. A vaccine or kit according to claim 13 where the mammal is a fur bearing animal including an animal selected from the group consisting of a *Canidae* species, a *Felidae* species, a mink, a chinchilla, a rabbit, a marten, a guinea pig and a racoon dog.

15. A vaccine or kit according to claim 13 where the mammal is a mammal selected from the group consisting of a ruminant, a horse, a swine and a man.

16. A vaccine or kit according to claim 1 comprising at least one further immunostimulating component.

17. A vaccine or kit according to claim 16 wherein the immunostimulating component is an adjuvant compound.

18. A vaccine or kit according to claim 17 where the adjuvant is selected from the group consisting of an oil-in-water emulsion, a water-in-oil emulsion, an aluminum compound, a synthetic polymer, a liposome and a cytokine.

19. A vaccine or kit according to claim 1 where at least one component is lyophilised.

20. A vaccine or kit according to claim 1 wherein at least one of the at least one first fungal species and the at least one second fungal species is suspended in a liquid phase.

21. A vaccine or kit according to claim 20 where the material of the at least one first fungal species comprises about 10^2-10^{10} microconidia of said species per ml.

22. A vaccine or kit according to claim 20 where the material of the at least one second fungal species comprises about 10^2-10^{10} microconidia of said species per ml.

23. A vaccine or a kit according to any of claims 1-22 where the material of the at least one first fungal species is live or viable.

24. A fungal strain which is selected from the group consisting of *Trichophyton equinum* strain DSM No. 13018, *Microsporum canis* DSM No. 13016, and *Microsporum canis* DSM No. 13017.

25. A method of manufacturing a vaccine or kit according to claim 1, the method comprising selecting an immunostimulating material of at least one first fungal species that is not pathogenic to the vertebrate animal, selecting at least one second fungal species which, in combination with said at least one first fungal species, is capable of conferring immune protection in said animal against said fungal skin infection, and combining said at least one first fungal species and said at least one second fungal species to obtain the vaccine or the kit.

26. A method according to claim 25 wherein the at least second fungal species is inactivated prior to being combined with said at least one first fungal species.

27. A method according to claim 25 wherein the at least second fungal species is inactivated by subjecting it to a heat treatment substantially without destroying its antigenic properties.

28. A method according to claim 27 wherein the at least one second fungal species is subjected to a heat treatment at a temperature within the range of about 40-60°C, including the range of about 45-55°C such as the range of about 48-52°C, and for a period of time within the range of about 30-1440 min such as the range of about 45-120 min, including about 60 min.

29. A method of preventing or curing a fungal skin infection in a vertebrate animal, the method comprising administering an effective dosage regimen of the vaccine or kit according to claim 1.

30. A method according to claim 29 wherein the animal is a mammal.

31. A method according to claim 30 wherein the mammal is a fur bearing animal including an animal selected from the group consisting of a *Canidae* species, a *Felidae* species, a mink, a chinchilla, a rabbit, a marten, a guinea pig and a racoon dog.

32. A method according to claim 30 wherein the mammal is a mammal selected from the group consisting of a ruminant, a horse, a swine and a man.

33. A method of producing an inactivated material of a fungal species that is capable of conferring immune protection in a vertebrate animal against a fungal skin infection, the method comprising a step of subjecting a live material of said fungal species to a heat treatment at a temperature and for a period of time that is sufficient to inactivate the fungal material substantially without destroying its antigenic properties.

34. A method according to claim 33 wherein the live material of said fungal species is subjected to a heat treatment at a temperature in the range of about 40-60°C, including the range of about 45-55°C such as the range of about 48-52°C, for a period of time in the

range of about 30-1440 min such as the range of about 45-120 min, including about 60 min.

35. A method according to claim 34 wherein the fungal species is selected from the group consisting of a *Microsporum* species, a *Trichophyton* species, a *Keratinomyces* species and an *Epidermophyton* species.

36. A vaccine or vaccine kit for the protection of a vertebrate animal against an infectious disease, said vaccine or kit comprising

(i) an effective amount of an immunostimulating material of at least one fungal species that is non-pathogenic to the vertebrate animal and

(ii) an immunologically effective amount of an antigenic material of at least one species of a pathogenic organism that, in combination with said material of the at least one fungal species, is capable of conferring immune protection in said animal against said infectious disease.

37. A vaccine or kit according to claim 36 where the at least one fungal species is a *Trichophyton* species.

38. A vaccine or kit according to claim 37 where the at least one fungal species is *Trichophyton equinum* including the strain DSM No. 13018.

39. A vaccine or kit according to claim 36 where the antigenic material of at least one species of a pathogenic organism is selected from the group consisting of material of a bacterial, viral, fungal or protozoan species.

40. A vaccine or kit according to claim 39 where the antigenic material is a material selected from the group consisting of viable whole cells, attenuated whole cells, inactivated whole cells and parts of any of said species comprising at least one epitope.

41. A vaccine or a kit according to any of claims 36-40 where the material of the at least one first fungal species is live or viable.

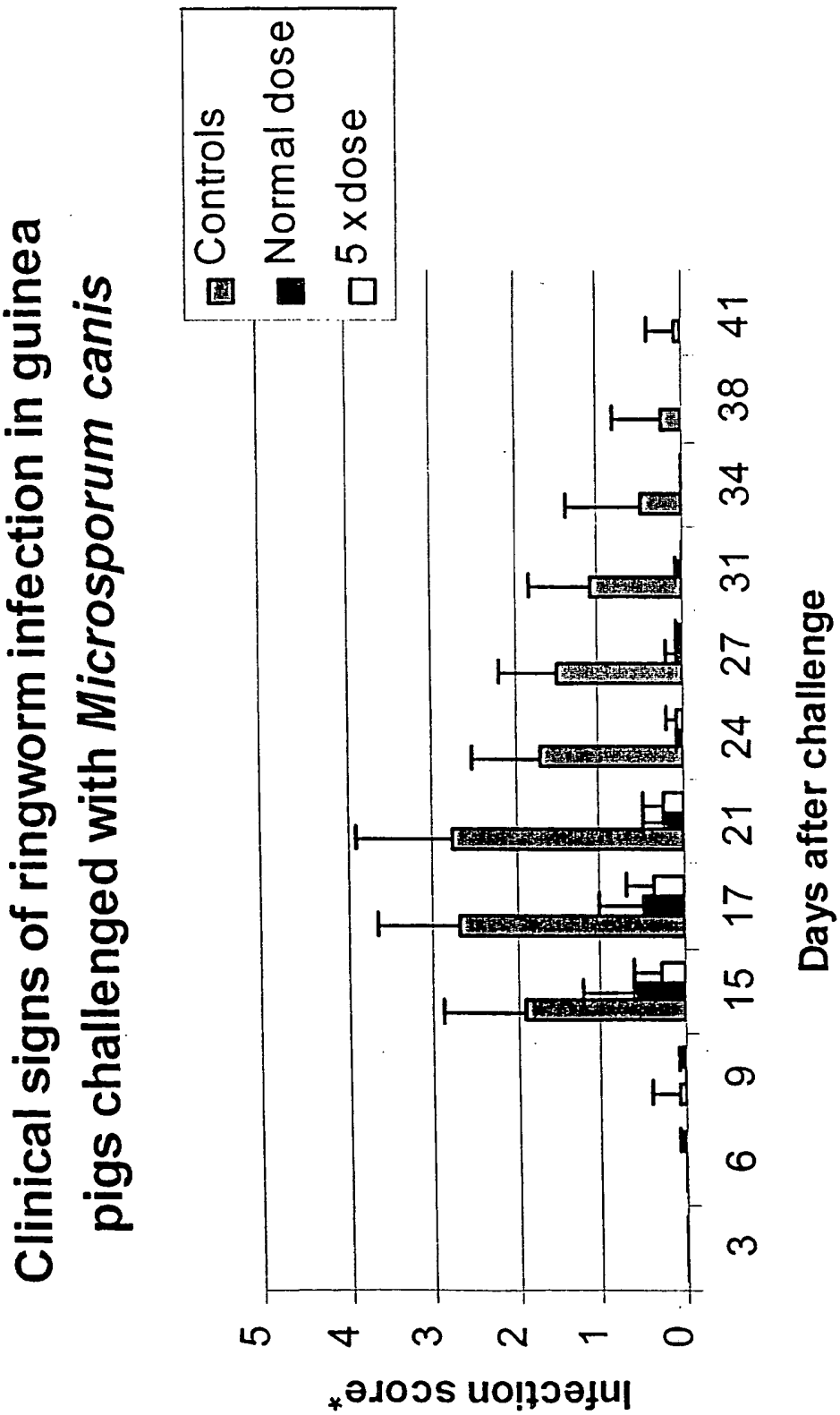
Clinical signs of ringworm infection in guinea pigs challenged with *Microsporum canis*
Controls
Normal dose
5 x dose
Infection score*
0
1
2
3
4
5
3
6
9
15
17
21
24
27
31
34
38
41
Days after challenge

Fig. 1

INTERNATIONAL SEARCH REPORT

Interna. Application No
PCT/IB 00/01196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/00 A61P17/00 A61P31/10 //C12N1/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE, LIFESCIENCES, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 15284 A (BOEHRINGER INGELHEIM VETMED ;BOEHRINGER INGELHEIM INT (DE); IVANOV) 16 April 1998 (1998-04-16) page 2, line 17 -page 4, line 24	1-4, 10-18, 20-22, 25,26, 29-32, 36-40
Y		5,19,23, 27,28,41

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2000	15/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Teyssier, B

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INTERNATIONAL SEARCH REPORT

Interna. . Application No
PCT/IB 00/01196

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 07894 A (BOEHRINGER INGELHEIM VETMED) 29 April 1993 (1993-04-29) page 3, line 26 -page 10; tables 8,9	1-4,6-9, 11-15, 20-22, 24-26, 29-32, 36-40
Y	---	5,16-19, 23,27, 28,41
Y	DATABASE WPI Section Ch, Week 199810 Derwent Publications Ltd., London, GB; Class B04, AN 1998-108811 XP002138790 & RU 2 084 241 C (GOLOVINA N P), 20 July 1997 (1997-07-20) abstract	16-18
Y	---	19
	DATABASE WPI Section Ch, Week 198518 Derwent Publications Ltd., London, GB; Class B04, AN 1985-108785 XP002138791 & SU 955 570 A (VETERINARY CPDS RES), 7 November 1984 (1984-11-07) abstract	
Y	---	23,41
	DATABASE WPI Section Ch, Week 199510 Derwent Publications Ltd., London, GB; Class B04, AN 1995-067723 XP002138792 & CZ 279 159 B (CHUMELA J), 18 January 1995 (1995-01-18) abstract	
X	---	33-35
	CARDENAS-FREYTAG L ET AL: "Effectiveness of a vaccine composed of heat-killed Candida albicans and a novel adjuvant, LT(R192G), against systemic candidasis" INFECTION AND IMMUNITY, vol. 67, no. 2, February 1999 (1999-02), pages 826-833, XP000857188	
Y	* page 827, "Vaccine preparation" *	5,27,28

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INTERNATIONAL SEARCH REPORT

Interna. . Application No
PCT/IB 00/01196

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 199224 Derwent Publications Ltd., London, GB; Class B04, AN 1992-198096 XP002138051 & SU 1 676 629 A (VETERINARY PREPARATIONS RES INST), 15 September 1991 (1991-09-15) abstract	36-41
A	DATABASE WPI Section Ch, Week 199831 Derwent Publications Ltd., London, GB; Class B04, AN 1998-360619 XP002138052 & RU 2 097 065 C (GOLOVINA N P), 27 November 1997 (1997-11-27) cited in the application abstract	1-41
A	DATABASE WPI Section Ch, Week 199825 Derwent Publications Ltd., London, GB; Class B04, AN 1998-180007 XP002138829 & CZ 283 615 B (BIOVETA AS), 13 May 1998 (1998-05-13) abstract	33-35
A	VILCINSKAS A ET AL: "Effect of the entomopathologic fungus Beauveria bassiana on the humoral immune response of Galleria mellonella larvae" EUROPEAN JOURNAL OF ENTOMOLOGY, vol. 94, 1997, pages 461-472, XP000913445 "Fungi and bacteria", pages 462-463	33-35

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
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